Electron Microscopy of Living Cells During *in-situ* Fluorescence Microscopy

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KEYWORDS

Scanning Electron Microscopy, Live Cell Imaging, Fluorescence Microscopy, Quantum Dots, Epidermal Growth Factor

Supplemental Information

Uptake and Transport of EGF bound QDs

The uptake and intra-cellular transport of EGF bound QDs in cells cultured on the silicon nitride membranes was initially investigated using commercial, stand-alone light microscopy. Cos7 cells were grown for one day in serum-free medium on glass slides (MatTek). Live staining of microtubules was performed by incubating the cells for 30 minutes in 100nM Tubulin-Tracker solution (Invitrogen). Cells were then labelled with 10nM EGF-QDOTs for 5 minutes, and imaged with a confocal scanning laser microscope (LSM510).

The motion of EGF-QD clusters during and after EGFR mediated internalization by Cos7 cells was characterized by live cell imaging with a confocal fluorescence and differential interference contrast (DIC) microscope. We also labelled the microtubules with a live staining reagent and analyzed the respective cellular distribution of the EGF-QD clusters and of the microtubule cytoskeleton, at different time points after labelling the cells.

We observed that during the first 15 minutes following the labelling, EGF-QDs were gradually taken up at the filopodia and transported in clusters along filopodia in the direction of the cell (Figure S1), as also previously reported by Lidke *et al.*[1]. After 15 minutes, clusters transported from the filopodia towards the cell body were observed to assemble and remain in the cell periphery at the outline of the microtubule network before being internalized into the cell body. This so-called “docking region” forms a distinct boundary surrounding the microtubule network (Figure S1b). After being internalized into the cell region containing the microtubule network, clusters followed the microtubules during transport towards the cell nucleus (Figure S1c). Clusters moving along tubules show directionally consistent movement. Collisions between

moving clusters result in temporary pauses in their transport, followed by either each cluster continuing its own path, or fusion into a larger cluster that also moves along the microtubule network[2]. In the ODEM experiments, we focused on uptake of the EGF-QDs and their transport along filopodia to the docking region.

Supplemental References

(1) Lidke, D. S.; Lidke, K. a; Rieger, B.; Jovin, T. M.; Arndt-Jovin, D. J. Reaching out for Signals: Filopodia Sense EGF and Respond by Directed Retrograde Transport of Activated Receptors. *J. Cell Biol.* **2005**, *170*, 619–626.

(2) Bálint, Š.; Verdeny Vilanova, I.; Sandoval Álvarez, Á.; Lakadamyali, M. Correlative Live-Cell and Superresolution Microscopy Reveals Cargo Transport Dynamics at Microtubule Intersections. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 3375–3380.

Supplemental Figure S1

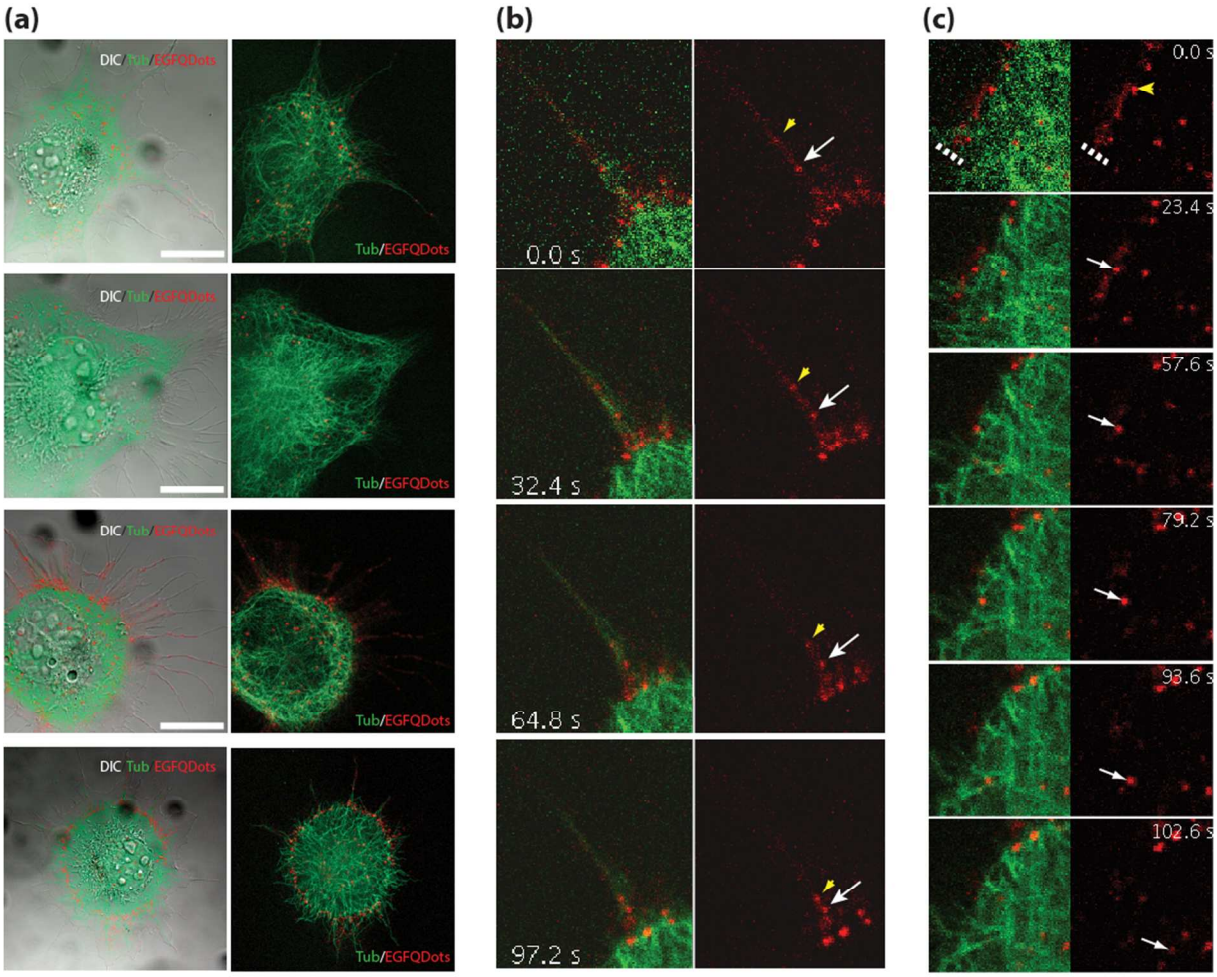
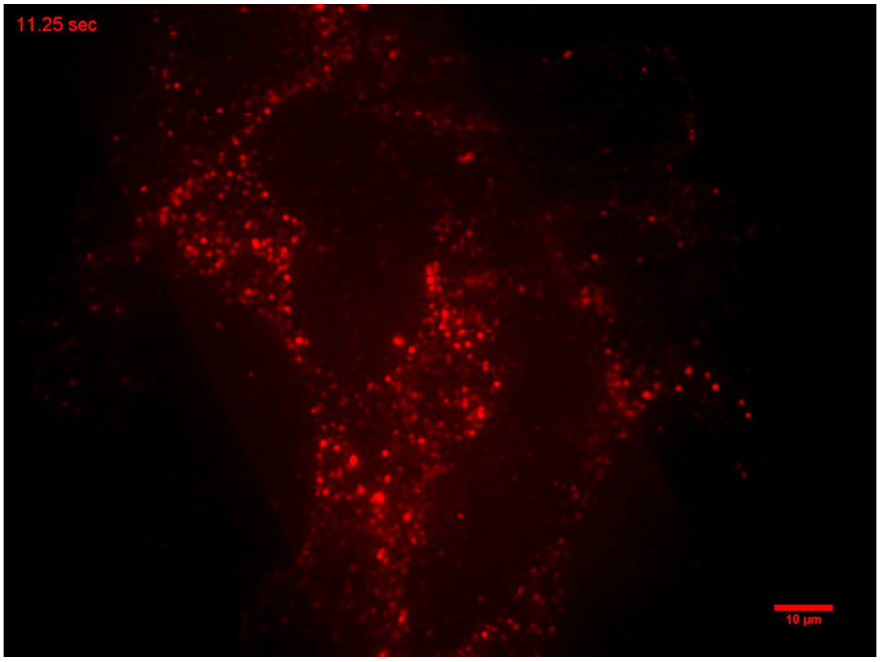


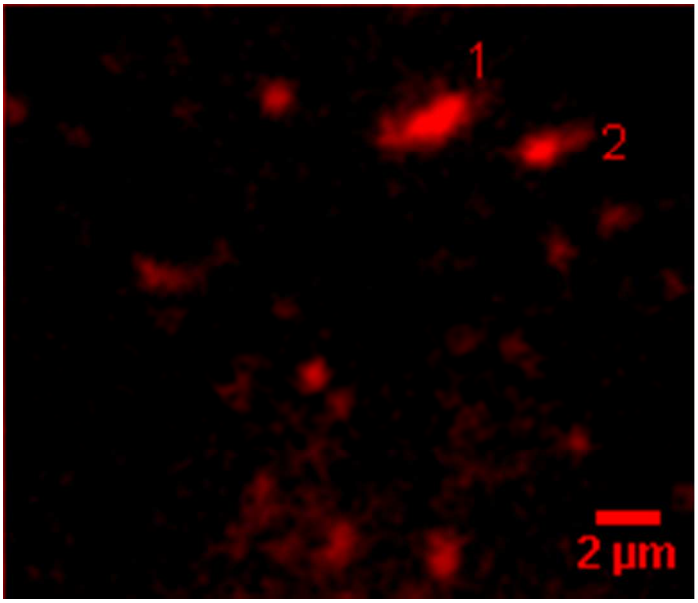
Figure S1. Various stages of EGF-QD uptake and transport. **(a)** EGF-QD uptake and transport in COS7 cells. The cells were grown for 1day in serum-free medium on glass slides. Live staining of microtubules was performed by Tubulin-Tracker and.the cells were further labelled with 10nM EGF-QDs for 5 minutes, and imaged with a confocal scanning laser microscope. Scale bars are 20 µm. **(b)** Time-lapse imaging of a similarly labelled living Cos7 cell zoomed in a filopodial extension. Arrows depict QDs filled vesicles which are uptaken in filopodia and being transported towards the cellular cortex. **(c)** Time-lapse imaging zoomed in cellular cortex and the microtubule frontier. Arrows depict EGF-QD conjugates which await at the microtubule frontier before being further transported in the cytoplasm.

Still image for Supplementary Video 1



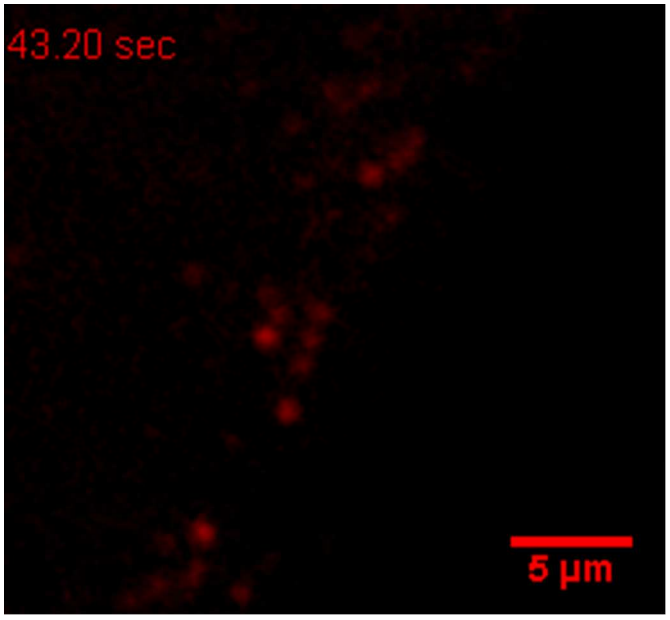
Fluorescent imaging of fibroblasts in liquid enclosure- the whole membrane area.

Still image for Supplementary Video 2



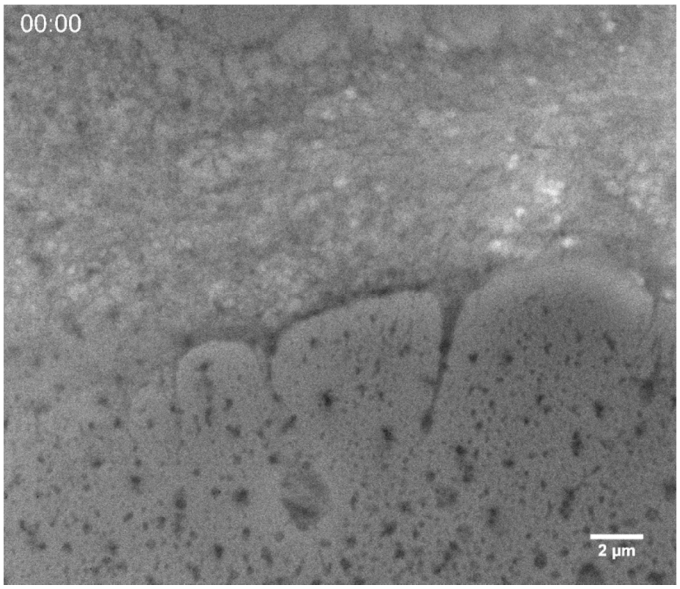
Fluorescent imaging of fibroblasts in liquid enclosure- the selected region in **Figure 2**, also shown in the time-lapse images.

Still image for Supplementary Video 3



Fluorescent imaging of fibroblasts in liquid enclosure- the selected region in **Figure 4,** also shown in the time-lapse images.

Still image for Supplementary Video 4



SEM video showing the detachment of cells after long exposure of the electron beam.